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Nephrotoxicity of Bence Jones proteins in the rat: Importance of protein isoelectric point

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Although Bence Jones proteins have been recognized for well over 100 years, it was not until 1962 that Edelman and Gally [1] demonstrated these proteins to be in fact the light polypeptide chains of all immunoglobulin classes. Strictly speaking, the term "Bence Jones protein" is reserved for those proteins identified by their solubility properties on heating [2]. These properties, however, have been shown to be somewhat nonspecific [3], and the term is now commonly used to denote appreciable amounts of, usually monoclonal, light chain appearing in the urine or serum of patients with multiple myeloma, primary amyloidosis [4], and monoclonal gammopathies [5]. Such light chains of mol wt of approximately 22,000 daltons commonly exist in dimer form in aqueous solution [1]. Bence Jones proteinuria is reported in various studies as being present in 40 to 80% of patients with myeloma [6, 7] and as being almost invariably present in 25% of patients with light-chain myeloma, in over 90% of patients with rarely occurring IgD-myeloma, in 35% of patients with IgG-myeloma, and 20% of those with IgA-myeloma [7].

Immunoglobulin light chains are normally filtered at the glomerulus and then catabolized in renal tubular epithelium [8–11]. Experiments both in man and mice with normal renal function have shown that when small doses of labeled light chain are injected, 1% or less is excreted in the urine [10, 11]. Waldmann et al [11] have estimated that 5 mg/kg/day of λ light chain is filtered in the normal human, with 0.04 mg/kg/day being excreted in the urine. Immunohistologic studies have shown that the vast majority of light chain is reabsorbed by proximal tubular epithelium [12]. Therefore, under normal circumstances, only minute amounts of light chain are presented to the distal nephron. In conditions such as multiple myeloma, macroglobulinemia, monoclonal gammopathy, and amyloidosis, very large amounts of light chain or Bence Jones protein may appear in the urine. It would seem that, in such situations, the capacity of the tubular epithelium to reabsorb and catabolize light chain is exceeded. Quantitative data on the capacity of proximal tubules to reabsorb light chain when the filtered load is large are lacking, though previous experiments from our laboratory have shown that about 20% of a dose of 300 mg κ chain injected i.p. in the rat was excreted in the urine over the first 24 hours and none subsequently [12].

There is now considerable clinical and experimental evidence to suggest that Bence Jones proteins, or at least some, are nephrotoxic. Cauchie et al [13] described a highly significant relationship between the presence of Bence Jones proteinuria and uremia, and there appears to be an unusually high incidence of Bence Jones proteinuria in patients with myeloma who develop episodes of acute renal failure. In a recent series and review of the literature by DeFronzo et al [14], 19 of 27 patients with myeloma and acute renal failure had Bence Jones proteinuria. The same group of investigators have also described greater impairment of creatinine clearance, PAH clearance, and urinary concentrating and acidifying ability in myeloma patients with Bence Jones proteinuria compared to those without [15]. A number of patients, however, continue to pass large amounts of Bence Jones protein in the urine for years with little or no impairment of renal function, suggesting that certain Bence Jones proteins may be more nephrotoxic than others.

Koss, Pirani, and Osserman [16] have shown that intraperitoneal injection of large amounts of one λ

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Bence Jones protein into mice produced a cast nephropathy with increase in serum urea nitrogen, though two other proteins tested under the same conditions failed to produce this effect. Previous studies from our laboratory failed to show any change in renal function apart from a rise in protein and albumin excretion in rats injected i.p. with 300 mg of one κ light chain, obtained from a patient who eventually died with moderate renal impairment. In vitro studies by Preuss, Hammack, and Murdaugh [17] suggest that Bence Jones proteins may inhibit proximal tubular organic anion transport and gluconeogenesis, though the purity of the preparations used here has been questioned [18].

To test the hypothesis that physical and chemical properties of certain individual Bence Jones proteins may be important in determining nephrotoxicity, we isolated and characterized Bence Jones proteins from 11 patients with myeloma and various degrees of renal dysfunction and tested them for acute nephrotoxicity in the aciduric, hydropenic rat.

Methods

Characterization of urinary proteins. Twenty-four-hour urine samples were collected from 11 patients with myeloma, Bence Jones proteinuria, and varying degrees of renal dysfunction. Urine was collected in ice with thymol added as a preservative. After removal of Tamm Horsfall mucoprotein, urine samples were concentrated with use of Amicon UM-10 membranes, dialyzed against distilled water, and analyzed by cellulose acetate electrophoresis [19] and immunoelectrophoresis [20] using antisera to whole human serum, κ and λ light chain, and Fc fragment. Six proteins were characterized as λ; and 5, as κ. The proteins were characfurther isolated by Sephadex G-75 gel filtration [21], and monomer and dimer fractions were collected. Both fractions were present in all but 4 patients, in whom only dimer was present.

Isoelectric focusing [22] was used to determine the isoelectric point (pI) of individual dimer fractions. A vertical pH gradient was set up and maintained in a density gradient made from a sucrose solution. The apparatus used was a LKB-8101 (LKB Produkter A.B., Stockholm Bronma, Sweden), and the 2 pH ranges used as Ampholine carrier electrolytes were 3 to 10 and 4 to 7. The protein content in the fractions isolated was measured by absorbancy at 280 nm. Pooled fractions were submitted to further characterization by immunodiffusion [23] and immunoelectrophoresis.

Protein secondary structure was examined by circular dichroism. Circular dichroism (CD) spectra were obtained with a Cary 61-CD spectopolarimeter and a 1-mm path length cuvette while flushing the optical path with nitrogen. The CD instrument was calibrated with an aqueous solution of *d*-10-camphorsulfonic acid (Aldrich) [24]. The proteins were studied at a concentration of 50 mg/ml in 20 mM potassium phosphate buffer (pH, 7.0). The analysis of spectra used here was novel for Bence Jones proteins in as far as the curve fitting was done using the STEPIT subroutine [25] in a Fortran program, HELIX, which describes the data within 95% confidence limits as estimated by the chi square goodness of fit criterion. This algorithm describes the CD spectrum using a three-component model consisting of fraction α helix, fraction β-pleated sheet, and a third category consisting of a total of all remaining secondary structure types. The standard ellipticity values are based on CD spectra obtained on proteins with known X-ray crystallographic structure [26].

In vivo studies. Female Sprague Dawley rats weighing 150 to 200 g were kept in metabolic cages with food withdrawn and with 1% ammonium chloride added to the drinking water for 24 hours prior to injection. Immediately prior to injection, rats were bled via the tail vein, and the bladder was emptied by manual compression. Urinary pH was determined on freshly voided urine. All rats with urine pH of 5.5 or less were then injected i.p. with 300 mg of test protein in 1 cc of normal saline. All protein solutions were urea-free and were clarified by centrifugation and passed through a no. 13 Millipore filter prior to injection. Groups of 3 rats were injected with Bence Jones protein dimer from each of the 11 patients. Sixteen rats were injected with equine myoglobin (pI, 6.8), 9 with ovalbumin (pI, 3.4), and 9 with bovine serum albumin. Rats were then kept in metabolic cages for 6 hours, with any urine passed being collected under mineral oil. Then, the bladder again was emptied. Rats then were sacrificed under sodium pentobarbital anesthesia, with renal tissue samples taken for ultrastructural and immunohistologic studies and with blood samples obtained by cardiac puncture. Blood samples were analyzed for serum urea nitrogen (kinetic urease method with IL 919 autoanalyzer) and creatinine (kinetic alkaline picrate method with IL 919 autoanalyzer) concentrations, and urine samples were analyzed for osmolality (freezing-point depression). Light chain content was determined by radial immunodiffusion.

Table 1. Clinical features and protein type in 11 patients with Bence Jones proteinuria

Patient no.	Disease[a]	Impaired renal function[b]	Acute renal failure[c]	Cast nephropathy
1	IgA κ myeloma	++	0	ND
2	LC κ myeloma	0	0	ND
3	IgG κ myeloma	+	0	0
4	IgD λ myeloma	+++	0	+++
5	IgA κ myeloma	0	0	ND
6	LC λ myeloma	+++	XI	+
7	LC λ myeloma	+++	XI	+
8	IgA λ myeloma	+++	0	ND
9	IgG λ myeloma	+++	0	ND
10	IgA κ myeloma	+++	0	+++
11	LC λ myeloma	+++	0	+++

[a] LC is light chain.
[b] Renal function is scored on lowest creatinine clearance during followup period. 0 = >80 ml/min, + = 50 to 80 ml/min, ++ = 20 to 50 ml/min, and +++ = < 20 ml/min.
[c] X1 = 1 episode.

To further investigate the effect of urine pH on nephrotoxicity, 7 rats were fed 6% sodium bicarbonate in the drinking water for 24 hours and then gavage fed 1 cc of 6% sodium bicarbonate 2 hours prior to injection so that the urine pH would be above 8. These rats were then injected i.p. with 300 mg of Bence Jones protein dimer, λ-type (pI, 6.2), from patient 9. Changes in renal function over 6 hours were compared with 7 aciduric rats injected with the same protein preparation.

Tissue for immunohistology was processed by the thin-section technique of Post [27], and sections incubated with fluorescein-conjugated rabbit or goat antisera to human κ and λ chain, Fc fragment, and rat albumin were examined with a Leitz Ortholux fluorescence microscope with appropriate filtration. Tissue for electron microscopy was processed as described previously [12], and ultrathin sections were examined with a Phillips 300 electron microscope.

Results

Protein characterization. The clinical details of the 11 patients from whom the urinary protein was isolated are shown in Table 1. Three patients (2, 3, and 5) had relatively normal renal function during their clinical course. Two patients (6 and 7) had episodes of acute renal failure following intravenous urography. In neither case was there recovery of renal function, and renal biopsy specimens obtained in both cases within 24 hours of the onset of oliguria revealed only minor distal cast formation. Three patients (4, 10, and 11) with chronically impaired renal function showed the characteristic cast nephropathy of myeloma kidney.

The protein characteristics are shown in Table 2. Five Bence Jones proteins were type κ, and six were type λ. In general, both monomer and dimer fractions were isolated by Sephadex G-75 chromatography except for one κ and three λ proteins where a dimer peak only was present. Protein isoelectric point ranged from 5.2 to 6.6. In general, patients with better renal function had Bence Jones protein of lower pI. Most proteins of higher pI associated with impaired renal function were type λ. The circular dichroism studies showed that, in general, κ light chains were of lower α helix content than were λ proteins, as has been shown by others. One κ protein (CD), however, had an extremely high α helix content. There did not seem to be a relationship between clinical renal impairment and protein secondary structure.

In vivo studies. Changes in serum urea nitrogen concentration in the 33 rats injected with the 11 Bence Jones protein dimer preparations of various

Table 2. Summary of protein characterization studies from 10 patients with Bence Jones proteinuria

Patient no.	Light chain type	Monomer/dimer	pI	Internal structure[a], %		
				α helix	β-sheet	Remainder
1	κ[b]	M, D	5.3	9.5 ± 0.5	24.5 ± 0.6	66.0
2	κ[b]	M, D	5.2	9.6 ± 0.4	28.5 ± 1.9	62.0
3	κ	D	5.4	59.9 ± 0.6	12.0 ± 2.1	28.1
4	λ	D	5.6	15.2 ± 0.6	23.1 ± 2.9	61.7
5	κ	M, D	5.8	13.8 ± 0.7	18.9 ± 4.3	67.3
6	λ	M, D	5.8	18.6 ± 0.5	20.9 ± 1.7	60.5
7	λ	D	6.2	15.5 ± 0.5	21.7 ± 0.6	62.8
8	λ	D	6.2	14.0 ± 0.2	30.8 ± 0.6	55.2
10	κ	M, D	6.6	13.2 ± 1.0	34.2 ± 2.1	52.6
11	λ	M, D	6.6	20.4 ± 2.2	24.0 ± 3.8	55.6

[a] Values are means ± 1 SD.
[b] Group 1

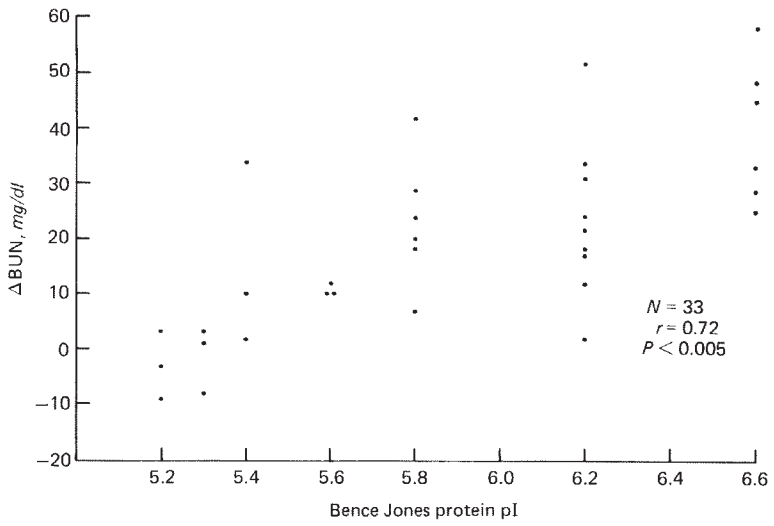

Fig. 1. *Change in serum urea nitrogen concentration* (ΔBUN) over 6 hours following i.p. injection of 300 mg of 11 different Bence Jones protein dimer preparations of pI 5.2 to 6.6 into 33 aciduric, hydropenic rats.

pI are shown in Fig. 1. There was a highly significant correlation ($P < 0.005$) between increase in serum urea nitrogen concentration over the 6-hour experimental period and the protein pI. Table 3 shows mean increase in urea nitrogen and serum creatinine concentrations in rats injected with Bence Jones protein (BJP) of pI less than 5.7, those of pI greater than 5.7, myoglobin, ovalbumin, and bovine serum albumin (BSA). Rats injected with six Bence Jones protein dimer preparations of pI greater than 5.7 had a mean rise in serum urea nitrogen of 28.0 mg/dl and in serum creatinine of 0.71 mg/dl, compared to rises of 5.3 mg/dl and 0.06 mg/dl, respectively, in rats injected with seven dimer preparations of pI greater than 5.7. Differences in values for change in serum urea nitrogen and creatinine when comparing these two groups were significant at the 1% level when analyzed by the Mann Whitney U test [28]. There was no significant difference between changes in serum creatinine and urea nitrogen when comparing rats injected with Bence Jones dimer of pI less than 5.7, myoglobin, ovalbumin, or bovine serum albumin.

Figure 2 shows light chain excretion rates over the 6-hour experimental period for 29 rats injected with the 11 Bence Jones protein dimer preparations compared to change in serum urea nitrogen. There was a wide variation in excretion rate, ranging from 2 μg/min to 200 μg/min, and a significant negative correlation ($P < 0.001$) between protein excretion rate and rise in urea nitrogen. In general, excretion rates of proteins of higher isoelectric point were less, but this phenomenon could not be separated from the greater rise in serum urea nitrogen in rats injected with these proteins. The mean excretion rate in 14 experiments where the injected protein was type κ was 23.9 ± (SEM) 1.46 μg/min, compared with a mean of 13.6 ± (SEM) 1.25 μg/min, in 15 experiments where type λ protein was injected. These values were not significantly different on analysis by the unpaired Student's *t*-test.

The 7 alkaluric rats (urine pH > 8) injected with type-λ Bence Jones protein dimer of pI 6.2 from patient 9 showed a mean rise of 1.8 mg/dl in serum urea nitrogen and 0.01 mg/dl in serum creatinine concentration over 6 hours, compared with a mean

Table 3. Mean rise in serum urea nitrogen (ΔSUN) and creatinine (ΔCreatinine) concentration over 6 hours for rats injected i.p. with 300 mg of test protein[a]

Group	Protein injected	pI	ΔSUN[b] *mg/dl*	ΔCreatinine[b] *mg/dl*
1	BJP dimer	<5.7	5.3 (*N* = 12) (−9 to +12)	0.06 (*N* = 10) (−0.19 to +0.41)
2	BJP dimer	>5.7	28.0[c] (*N* = 21) (+6 to +56)	0.71[c] (*N* = 18) (+0.05 to +1.78)
3	Myoglobin	6.8	5.2[d] (*N* = 16) (−4 to +12)	0.07[d] (*N* = 16) (−0.18 to +0.39)
4	Ovalbumin	4.3	1.2[d] (*N* = 9) (−8 to +7)	0.09[d] (*N* = 9) (−0.15 to +0.40)
5	BSA	4.9	0.0[d] (*N* = 9) (−10 to +7)	—

[a] Abbreviations used are: BJP, Bence Jones protein; BSA, bovine serum albumin; pI, isoelectric point; *N*, number of observations.
[b] Range is given in parentheses.
[c] $P < 0.01$ compared to group 1.
[d] NS, compared to group 1.

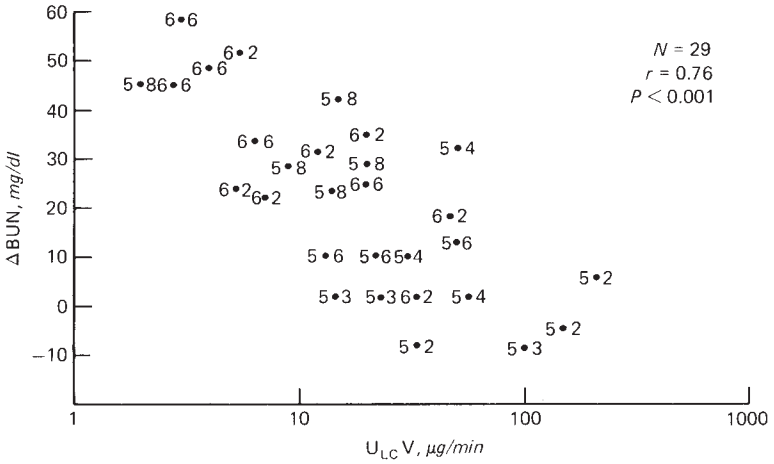

Fig. 2. *Comparison between light change excretion rates ($U_{LC}V$) and change in serum urea nitrogen concentration (ΔBUN) over 6 hours following i.p. injection of 300 mg of 11 different Bence Jones protein dimer preparations of pI 5.2 to 6.6 into 29 aciduric, hydropenic rats.*

rise of 19.3 mg/dl in urea nitrogen and 0.55 mg/dl in creatinine in 7 aciduric rats (urine pH < 5.5) injected with the same protein preparation (Table 4). Differences between these two groups were significant at the same level ($P = 0.009$) for both urea nitrogen and creatinine when analyzed by the Mann Whitney U test. Preinjection urine osmolality ranged from 1500 to 2200 mOsm in the aciduric group and 1800 to 2300 mOsm in the alkaluric group.

The morphologic studies, which will be reported in detail separately, showed proximal tubular droplets immunoreactive with the injected light chain type in all rats injected with Bence Jones protein. Electron-dense casts were seen in the distal convoluted tubule, cortical collecting tubule, and medullary collecting duct in 9 aciduric rats with acute deterioration of renal function. These casts were immunoreactive with antisera to the injected light chain type, but with less than 30% of medullary collecting ducts being involved on renal cross-section. No casts were seen in rats injected with Bence Jones protein preparations of pI < 6.2, in alkaluric rats, or in rats injected with ovalbumin or bovine serum albumin. Occasional casts were seen in 2 of 16 rats injected with myoglobin.

Table 4. Comparison of changes in serum urea nitrogen (ΔSUN) and serum creatinine (Δcreatinine) concentrations over 6 hours for aciduric and alkaluric rats injected with 300 mg Bence Jones protein dimer (pI, 6.2) from patient 9

Urine pH	*N*	ΔSUN[a] *mg/dl*	ΔCreatinine[a] *mg/dl*
<5.5	7	19.3[b] (−1 to +52)	0.55[b] (+0.05 to +1.34)
>8.0	7	1.8[b] (−2 to +7)	0.01[b] (−0.25 to +0.27)

[a] Values are expressed as mean, with range in parentheses.
[b] $P < 0.01$ for both ΔSUN and Δcreatinine.

Discussion

Protein characterization. Although renal dysfunction in patients with myeloma may be due to a wide variety of causes [7], only three manifestations seem to bear a direct relationship to the presence of Bence Jones proteinuria. These are (*1*) "myeloma kidney," a chronic cast nephropathy with gradual deterioration of renal function, (*2*) acute renal failure [7], and (*3*) a more rarely occurring adult Fanconi syndrome [29]. Possible factors that may determine Bence Jones protein nephrotoxicity include the total load of protein excreted, structural features determining ease of protein precipitation, isoelectric point and charge density of the protein, protein concentration in the tubular lumen, and tubular fluid pH.

Although the numbers are small it is notable that all 6 patients with λ light chain had severely impaired renal function compared with only 1 of 5 patients with κ light chain. This contrasts with the finding of Stone and Frenkel [30] that light chain type did not correlate with renal function in 35 patients with light chain myeloma in spite of the fact that the proteinuric rate for λ light chain has been shown to be 2.5 times that for κ [8]. It is interesting that in the two patients with light chain myeloma who developed acute renal failure after intravenous urography, cast formation was not a prominent feature on renal biopsy performed at the time of onset of the renal failure. This contrasts to most cases reported of acute renal failure in patients with myelo-

ma where moderate to severe cast formation has been present, although it was only a minor feature in 2 of 9 cases reported by DeFronzo et al [14].

The results of the circular dichroism studies indicate that, in general, the α helical content separates the λ- from the κ-type proteins. This is consistent with the prediction of Kincaid and Jurgensons [31] that λ-type Bence Jones proteins contain a greater α helical content than do the κ, based on ellipticity data near 19 nm. There is no obvious correlation between either β-pleated sheet content or remaining secondary structure content and immunologic type. With the one exception, the κ-type proteins range from 18.9 to 34.2% β-pleated sheet, and the λ proteins range from 20.9 to 30.8%. This is in the range predicted from X-ray [32] as well as earlier circular dichroism data [33]. No relationship between internal structure and nephrotoxicity was seen in this study, though other data from our laboratory show that three light chains that form crystal-like structures in proximal tubular epithelium are all κ group 1 of low α-helical content [34].

In general, there was more impairment of renal function if the Bence Jones protein isoelectric point was high. We are not aware of any published data that relates isoelectric point to renal function. One previously reported case [29] describes a patient with Fanconi syndrome, amyloidosis, and 10 year's proteinuria with κ chain of isoelectric point 6.2 who had no evidence of impaired GFR during followup. She did have renal tubular acidosis, however, and serial urine pH values were never below 5.5. Thus, her relative alkaluria may have proved a protective mechanism. It may be that conditions of aciduria or dehydration may have to be at least intermittantly present for Bence Jones proteins to exert a nephrotoxic effect.

In vivo studies. The experiments described here were performed in the aciduric, hydropenic rat as we postulated that these conditions would be most likely to enhance distal cast formation. The three control proteins were chosen for differences in filterability and isoelectric point. Bovine serum albumin (mol wt, 66,000 daltons; pI, 4.9) is poorly filtered at the glomerulus, and it is of low pI. Ovalbumin (mol wt, 44,000 daltons; pI, 4.3) is more readily filtered and is anionic under conditions of aciduria. Myoglobin (mol wt, 17,000; pI, 6.8) is readily filtered and is cationic under conditions of aciduria. Previous experiments using a κ-type Bence Jones protein produced no deterioration of renal function in the same type of rat with free access to water and urinary pH ranging from 5.8 to 7.3 [12]. The dose of protein selected here was the same as in the previous studies. Much higher doses may be necessary to produce a chronic cast nephropathy, as shown by Koss, Pirani, and Osserman [16]. In the experiments described by these workers, injection of 200 mg of a type-λ Bence Jones protein into mice was necessary to produce significant cast formation and elevation in blood urea nitrogen under conditions of free access to food and water.

Although a significant negative correlation is seen here between Bence Jones protein isoelectric point and urinary light chain excretion, the meaning of this finding is uncertain, as those rats injected with protein of higher isoelectric point had greater elevation of blood urea nitrogen and serum creatinine over the experimental period and presumably lower GFR. Also the failure to find a significant difference in excretion rates for κ- and λ-type proteins might be influenced by the fact that the λ proteins used here, in general, were of higher isoelectric point than the κ proteins. It is difficult to compare these data with that of Wochner, Strober, and Waldmann [8], who found the proteinuric rate in mice for human λ light chains was approximately two and one half that of κ, as the experimental conditions were so different with smaller doses of labeled light chains used.

The sharp deterioration of renal function seen here with some proteins over the 6-hour experimental period did not correlate with light chain type or internal structure but did show a significant correlation with the protein isoelectric point. The most marked elevation of blood urea nitrogen and serum creatinine concentrations was seen with proteins having an isoelectric point of 5.8 or greater. As the urinary pH was 5.5 or less, this finding suggests that these proteins may be more nephrotoxic when cationic than when anionic or when near the isoelectric point, where the tendency for the proteins to precipitate is greatest [35]. The mechanism of this nephrotoxicity cannot be explained by the present experiments. Other experiments in our laboratory [36] have shown that Bence Jones proteins, myoglobin, and hemoglobin coprecipitate in vitro with Tamm Horsfall mucoprotein as pH is lowered from 6.0 to 4.5. It may be that some interaction between the cationic Bence Jones proteins in the tubular lumen and the negatively charged Tamm Horsfall mucoprotein (pI, 3.5) which lines the distal nephron [37] may initiate reduction of GFR in some way. It does not seem likely that this is achieved via extensive tubular obstruction because cast formation was not that extensive in these experiments and was, in

fact, absent in some rats with acute deterioration in renal function. Also the failure of myoglobin (pI, 6.8) to induce acute deterioration of renal function in this model suggests that a simple charge interaction is not the sole mechanism, though myoglobinuria has long been associated with acute nephrotoxicity, and an early paper by Bywaters and Stead [38] described alkaluria as preventing acute deterioration of renal function in rabbits injected with myoglobin. Further, more precise measurements of various parameters of renal function during infusion of these proteins under conditions of varying urinary pH would seem necessary to further elucidate the mechanisms of this nephrotoxicity.

Summary

Bence Jones proteins (BJP) were isolated from the urine of 12 patients with multiple myeloma and various degrees of renal dysfunction. Proteins were characterized as to type (six type λ and five type κ), isoelectric point (pI), and secondary structure by circular dichroism (CD). Clinical renal function was more impaired with type-λ proteins and with proteins of pI > 5.7. CD studies distinguished κ from λ proteins in most cases but did not correlate with nephrotoxicity. Protein dimer preparations were tested for nephrotoxicity in aciduric, hydropenic, female, Sprague Dawley rats by following renal function and morphology over 6 hours after injection i.p. of 300 mg of protein. Twelve rats of urine pH < 5.5 injected with four BJP of pI < 5.7 showed a mean rise in SUN of 5.3 mg/dl and in creatinine of 0.06 mg/dl, compared with a mean rise of 28.0 mg/dl (SUN) and 0.75 mg/dl (creatinine) in 21 rats injected with seven BJP of pI > 5.7 ($P < 0.01$). Seven sodium-bicarbonate-fed rats of urine pH > 8 injected with a BJP of pI 6.2 showed mean rise in SUN of 1.8 mg/dl and in creatinine of 0.01 mg/dl, compared with 19.3 mg/dl (SUN) and 0.55 mg/dl (creatinine) in 7 aciduric rats injected with the same BJP ($P = 0.009$). Morphologic and immunohistologic studies showed distal cast formation in 9 rats with acute deterioration in renal function. It is concluded that BJP of pI > urine pH are acutely nephrotoxic in the rat by a mechanism that may involve a charge interaction in the distal nephron.

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